IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Patent Application of

INZÉ et al.I — Atty. Ref.: 5547-2; Confirmation No. 1234

Appl. No. 10/531,475 — TC/A.U. 1638

Filed: April 15, 2005 — Examiner: Collins

For: IDENTIFICATION OF NOVEL E2F TARGET GENES AND USE THEREOF

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Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Sir:

RULE 132 DECLARATION

I, Valerie Frankard, do hereby declare and say as follows:

1. I am an employee of CropDesign N.V., in Gent, Belgium, the Assignee of the above-identified application.

2. I have reviewed the above-identified application as well as the claims.

3. I am a Belgian citizen residing at Waterloo, Belgium.

4. From 1982-1987 I studied agronomical engineering and received a Master's Degree from the Université Libre de Bruxelles, in Brussels, Belgium. From 1987-1992, I studied Cell and Gene Biotechnology at the Vrije Universiteit Brussel, in Brussels, Belgium, where I earned my PhD degree. My thesis was in the field of plant nutritional quality improvement via biotechnology. From 1993-1999, I was a postdoctoral

researcher and principal investigator (P1) at the Vrije Universiteit Brussel, in Brussels, Belgium.

5. From 1999 to 2009, I have held the position of Technology Management Coordinator of CropDesign N.V., in Gent, Belgium.

6. I am presently Senior Scientist, Yield Biology, and Research Manager of the Rice Yield Project at CropDesign.

7. I have reviewed the results submitted to the U.S. Patent Office in the above in remarks presented July 13, 2009. The results are presented again herein, with corrections, as work that I am familiar with and which has been performed to demonstrate that the claimed invention is supported by a disclosure which teaches one of ordinary skill in the art how to make and use the claimed invention, without requiring undue experimentation.

8. The previously-presented results were reported as including SEQ ID NO:1835. It has now been appreciated that the previously-presented results were conducted with a sequence referred to in the following alignment as CDSxx77, which comprises one mismatch to SEQ ID NO: 1835 within the coding sequence

```
                            Needle SEQID1835-CDSxx77.txt
########################################
# Program: needle
# Rundate: Fri 26 Mar 2010 14:36:47
# Commandline: needle
#    -auto
#    -asequence /srv/www/vhosts/embossgui/htdocs/output/832812/.asequence
#    -bsequence /srv/www/vhosts/embossgui/htdocs/output/832812/.bsequence
#    -brief
#    -outfile outfile
#    -aformat3 srspair
# Align_format: srspair
# Report_file: outfile
########################################

#=======================================
#
# Aligned_sequences: 2
# 1: CDSxx77
# 2: SEQID1835
# Matrix: EDNAFULL
# Gap_penalty: 10.0
# Extend_penalty: 0.5
#
# Length: 982
# Identity:     821/982 (83.6%)
# Similarity:   821/982 (83.6%)
# Gaps:         160/982 (16.3%)
# Score: 4101.0
#
#
#=======================================

CDSxx77            1 gtgcacaattgatgagcaatgctttttataatgccaactttgtacaaaa     50

SEQID1835          0 --------------------------------------------------      0

CDSxx77           51 aagcaggcttaaacaatggccctcgaagcgatgaacactccaacttcttc    100
                                 ||||||||||||||||||||||||||||||||||||
SEQID1835          1 --------------atggccctcgaagcgatgaacactccaacttcttc     35

CDSxx77          101 tttcaccagaatcgaaacgaaagaagatttgatgaacgacgccgttttca    150
                     ||||||||||||||||||||||||||||||||||||||||||||||||||
SEQID1835         36 tttcaccagaatcgaaacgaaagaagatttgatgaacgacgccgttttca     85

CDSxx77          151 ttgagccgtggcttaaacgcaaacgctccaaacgtcagcgttctcacagc    200
                     ||||||||||||||||||||||||||||||||||||||||||||||||||
SEQID1835         86 ttgagccgtggcttaaacgcaaacgctccaaacgtcagcgttctcacagc    135

CDSxx77          201 ccttcttcgtcttcttcctcaccgcctcgatctcgacccaaatcccagaa    250
                     ||||||||||||||||||||||||||||||||||||||||||||||||||
SEQID1835        136 ccttcttcgtcttcttcctcaccgcctcgatctcgacccaaatcccagaa    185

CDSxx77          251 tcaagatcttacggaagaagagtatctcgctctttgtctcctcatgctcg    300
                     ||||||||||||||||||||||||||||||||||||||||||||||||||
SEQID1835        186 tcaagatcttacggaagaagagtatctcgctctttgtctcctcatgctcg    235

CDSxx77          301 ctaaagatcaaccgtcgcaaacgcgatttcatcaacagtcgcaatcgtta    350
                     ||||||||||||||||||||||||||||||||||||||||||||||||||
SEQID1835        236 ctaaagatcaaccgtcgcaaacgcgatttcatcaacagtcgcaatcgtta    285

CDSxx77          351 acgccgccgccagaatcaaagaaccttccgtacaagtgtaacgtctgtga    400
                     ||||||||||||||||||||||||||||||||||||||||||||||||||
SEQID1835        286 acgccgccgccagaatcaaagaaccttccgtacaagtgtaacgtctgtga    335
                                 Page 1
```

Needle SEQID1835-CDSxx77.txt

```
CDSxx77          401 aaaagcgtttccttcctatcaggctttaggcggtcacaaagcaagtcacc    450
                     ||||||||||||||||||||||||||||||||||||||||||||||||||
SEQID1835        336 aaaagcgtttccttcctatcaggctttaggcggtcacaaagcaagtcacc    385

CDSxx77          451 gaatcaaaccaccaaccgtaatctcaacaaccgccgatgattcaacagct    500
                     ||||||||||||||||||||||||||||||||||||||||||||||||||
SEQID1835        386 gaatcaaaccaccaaccgtaatctcaacaaccgccgatgattcaacagct    435

CDSxx77          501 ccgaccatctccatcgtcgccggagaaaaacatccgattgctgcctccgg    550
                     ||||||||||||||||||||||||||||||||||||||||||||||||||
SEQID1835        436 ccgaccatctccatcgtcgccggagaaaaacatccgattgctgcctccgg    485

CDSxx77          551 aaagatccacgagtgttcaatctgtcataaagtgtttccgacgggtcaag    600
                     ||||||||||||||||||||||||||||||||||||||||||||||||||
SEQID1835        486 aaagatccacgagtgttcaatctgtcataaagtgtttccgacgggtcaag    535

CDSxx77          601 ctttaggcggtcacaaacgttgtcactacgaaggcaacctcggcggcgga    650
                     ||||||||||||||||||||||||||||||||||||||||||||||||||
SEQID1835        536 ctttaggcggtcacaaacgttgtcactacgaaggcaacctcggcggcgga    585

CDSxx77          651 ggaggaggaggaagcaaatcaatcagtcacagtggaagcgtgtcgagcac    700
                     ||||||||||||||||||||||||||||||||||||||||||||||||||
SEQID1835        586 ggaggaggaggaagcaaatcaatcagtcacagtggaagcgtgtcgagcac    635

CDSxx77          701 ggtatcggaagaaaggagccaccgtggattcatcgatctaaacctaccgg    750
                     ||||||||||||||||||||||||||||||||||||||||||||||||||
SEQID1835        636 ggtatcggaagaaaggagccaccgtggattcatcgatctaaacctaccgg    685

CDSxx77          751 cgttacctgaactcagccttcatcacaatccaatcgtcgacgaagagatc    800
                     ||||||||||||||||||||||||||||||||||||||||||||||||||
SEQID1835        686 cgttacctgaactcagccttcatcacaatccaatcgtcgacgaagagatc    735

CDSxx77          801 ttgagtccgttgaccggtaaaaaaccgcttttgttgaccgatcacgacca    850
                     ||||||||||||||||||||||||||||||||||||||||||||||||||
SEQID1835        736 ttgagtccgttgaccggtaaaaaaccgcttttgttgaccgatcacgacca    785

CDSxx77          851 agtcatcaagaaagaagatttttctttaaaaatctaatactcgaacccag    900
                     ||||||||||||||||||||.||||||||||||||||
SEQID1835        786 agtcatcaagaaagaagatttatctttaaaaatctaa----------    822

CDSxx77          901 ctttcttgtacaaagttggcattataagaaagcattgcttatcaatttgt    950

SEQID1835        822 --------------------------------------------------    822

CDSxx77          951 tgcaacgaacaggtcacttcatcaataatatc    982

SEQID1835        822 -------------------------------    822
```

9. The peptide encoded by CDSxx77 is 99.6% similar to the peptide encoded by SEQ ID NO:1835 (i.e., SEQ ID NO:1836) as demonstrated by the following alignment:

```
                            Needle SEQID1836-CDSxx77.txt
########################################
# Program:  needle
# Rundate:  Wed Feb 13 16:08:53 2008
# Align_format: srspair
# Report_file: outfile
########################################
#=======================================
#
# Aligned_sequences: 2
# 1: SEQID1836
# 2: CDSxx77p
# Matrix: EBLOSUM62
# Gap_penalty: 11.0
# Extend_penalty: 1.0
#
# Length: 273
# Identity:     272/273 (99.6%)
# Similarity:   272/273 (99.6%)
# Gaps:           0/273 ( 0.0%)
# Score: 1427.0
#
#
#=======================================

SEQID1836          1 MALEAMNTPTSSFTRIETKEDLMNDAVFIEPWLKRKRSKRQRSHSPSSSS     50
                     ||||||||||||||||||||||||||||||||||||||||||||||||||
CDSxx77p           1 MALEAMNTPTSSFTRIETKEDLMNDAVFIEPWLKRKRSKRQRSHSPSSSS     50

SEQID1836         51 SSPPRSRPKSQNQDLTEEEYLALCLLMLAKDQPSQTRFHQQSQSLTPPPE    100
                     ||||||||||||||||||||||||||||||||||||||||||||||||||
CDSxx77p          51 SSPPRSRPKSQNQDLTEEEYLALCLLMLAKDQPSQTRFHQQSQSLTPPPE    100

SEQID1836        101 SKNLPYKCNVCEKAFPSYQALGGHKASHRIKPPTVISTTADDSTAPTISI    150
                     ||||||||||||||||||||||||||||||||||||||||||||||||||
CDSxx77p         101 SKNLPYKCNVCEKAFPSYQALGGHKASHRIKPPTVISTTADDSTAPTISI    150

SEQID1836        151 VAGEKHPIAASGKIHECSICHKVFPTGQALGGHKRCHYEGNLGGGGGGGS    200
                     ||||||||||||||||||||||||||||||||||||||||||||||||||
CDSxx77p         151 VAGEKHPIAASGKIHECSICHKVFPTGQALGGHKRCHYEGNLGGGGGGGS    200

SEQID1836        201 KSISHSGSVSSTVSEERSHRGFIDLNLPALPELSLHHNPIVDEEILSPLT    250
                     ||||||||||||||||||||||||||||||||||||||||||||||||||
CDSxx77p         201 KSISHSGSVSSTVSEERSHRGFIDLNLPALPELSLHHNPIVDEEILSPLT    250

SEQID1836        251 GKKPLLLTDHDQVIKKEDLSLKI    273
                     ||||||||||||||||.||||
CDSxx77p         251 GKKPLLLTDHDQVIKKEDFSLKI    273
```

10. In view of this similarity, I believe that the previously presented results demonstrate that one of ordinary skill in the art could make and use the claimed invention without undue experimentation. The error in previously identifying the sequence of the previously presented results as SEQ ID NO:1835 occurred without deceptive intent.

11. <u>Example A: CDSxx77 under the control of the constitutive promoter GOS2</u>

A DNA fragment encoding the 2XC2H2 protein represented in the application by CDSxx77p was isolated from an *Arabidopsis thaliana* seedling cDNA library (Invitrogen, Paisley, UK). PCR was performed using Hifi Taq DNA polymerase in standard conditions, using 200 ng of template in a 50 µl PCR mix. The primers used for the PCR amplification (and which include the AttB sites for Gateway recombination) were as follows:

Forward primer: ggggacaagtttgtacaaaaaagcaggcttaaacaatggccctcgaagcg

Reverse primer: ggggaccactttgtacaagaaagctgggttcgagtattagatttttaaagataaatc

The amplified PCR fragment was purified also using standard methods. The first step of the Gateway procedure, the BP reaction, was then performed, during which the PCR fragment recombined *in vivo* with the pDONR201 plasmid to produce, according to the Gateway terminology, an "entry clone". Plasmid pDONR201 was purchased from Invitrogen, as part of the Gateway® technology.

The entry clone comprising CDSxx77 was then used in an LR reaction with a destination vector used for *Oryza sativa* transformation. This vector contained as functional elements within the T-DNA borders: a plant selectable marker; a screenable marker expression cassette; and a Gateway cassette intended for LR *in vivo* recombination with the nucleic acid sequence of interest already cloned in the entry clone. A rice GOS2 promoter for constitutive specific expression was located upstream of this Gateway cassette. After the LR recombination step, the resulting expression vector (pGOS2::CDSxx77) was transformed into *Agrobacterium* strain LBA4044 according to methods well known in the art.

Rice transformation and phenotypic evaluation of the plants was as described in Example 12.

The results are shown in Table I below.

Table I: Results of phenotypic characterization of rice plants transformed with pGOS2::CDSxx77. Overall percentage of increase is given for biomass (above ground area and root area), total seed number and for flowers per panicle for T1 and T2 plants.

	T1 generation		T2 generation	
Parameter	% increase	p-value	% increase	p-value
Above ground area	10.5	0.002	9.5	0.000
Root area	7.4	0.01	4.5	0.0008
Plant height	5.5	0.000	2.8	0.000
Total number of seeds	9.7	0.031	11.9	0.001
Flowers per panicle	7.8	0.000	8.0	0.001

In addition, an increase was observed for seed fill rate (3 positive lines out of 4 in T2, overall increase of 30.1% with a p-value of 0.000), for harvest index (3 positive lines out of 4 in T2, overall increase of 35.0% with a p-value of 0.000) and for thousand kernel weight (2 positive lines out of 4 in T2, overall increase of 1.9% with a p-value of 0.005).

12. Example B: CDSxx77 under the control of the seed-specific promoter prolamin

Cloning of CDSxx77 was as described above. The entry clone was subsequently used in an LR reaction with a destination comprising the seed-specific prolamin promoter (mentioned in Example 12). Plant transformation and phenotypic analysis were as described above and as described in Example 12.

Table II: Results of phenotypic characterization of rice plants transformed with pPROLAMIN:: CDSxx77.

	T1 generation		T2 generation	
Parameter	% increase	p-value	% increase	p-value
Early vigour	25.7	0.001	10.4	0.021
Total seed weight	17.7	0.001	8.3	0.007
Total number of seeds	11.0	0.003	5.1	0.049
Seed fill rate	7.4	0.014	1.9	0.123
Harvest index	14.3	0.000	5.1	0.040
Number of filled seeds	19.5	0.000	7.2	0.018

In addition, an increase was observed for thousand kernel weight (2 positive lines out of 4 in T2, with respectively an increase of 3.1% with a p-value of 0.019, and an increase of 3.2% with a p-value of 0.013).

13. I believe that the above demonstrates that the claims are supported by a disclosure which teaches one of ordinary skill in the art how to make and use the claimed invention, without requiring undue experimentation.

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false

statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

Signed this 21 day of May, 2010.

(Signature) _____
Valerie Frankard